

Synchronisation of stochastic oscillators in biochemical systems

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A formalism is developed which describes the extent to which stochastic oscillations in biochemical models are synchronised. It is based on the calculation of the complex coherence function within the linear noise approximation. The method is illustrated on a simple example and then applied to study the synchronisation of chemical concentrations in social amoeba. The degree to which variation of rate constants in different cells and the volume of the cells affects synchronisation of the oscillations is explored, and the phase lag calculated. In all cases the analytical results are shown to be in good agreement with those obtained through numerical simulations.

PACS numbers: 05.40.-a, 87.18.Tt, 87.10.Mn

I. INTRODUCTION

Oscillatory behaviour is observed in a great variety of biochemical systems, over a wide range of time periods [1, 2]. These oscillations have been modelled extensively, using both deterministic and stochastic frameworks [3–6]. Most of this modelling is carried out at the single cell level. However, many processes display coherent behaviour over a population of cells. This requires that the oscillations in the individual cells must influence each other. One reason for this is that random fluctuations, intrinsic to these systems, can introduce random phase lags. This means that a population of isolated cells demonstrating oscillatory dynamics will not remain synchronised over time, even if they are synchronised initially. Therefore, for coherent, collective behaviour (such as intercellular signalling), some form of communication between the cells is necessary.

Before proceeding further we need to discuss what is meant by the term ‘synchronisation’. This is necessary to do this since its precise definition varies. For the stochastic time series of the kind that will interest us here, the approach of Pikovsky, Roseblum & Kurths will be the most relevant. They define synchronisation as “an adjustment of rhythms of oscillating objects due to their weak interaction” [7]. Using reaction systems as examples, we will show the forms that the interaction can take, and what ‘weak’ means in this context.

One place in which the role and mechanisms of synchronisation have been systematically analysed is the study of the dynamics of glycolytic metabolism. Richard *et al.* have performed experiments in yeast cells, and observed oscillations both in individual cells and populations of cells [8, 9]. The authors conclude that the coupling of the cells *via* an extracellular metabolite (proposed to be acetaldehyde) causes the oscillations of the individual cells to synchronise. In [9], two cell popula-

tions, originally oscillating 180° out of phase, became synchronised upon coupling, and a time to synchronise was measured.

Several mathematical models have attempted to capture and explain this behaviour. One of these is due to Wolf & Heinrich, devised in [10] and refined in [11]. They begin by describing a deterministic, one-cell model, where the cell is connected to an extra-cellular compartment, which exhibits either a stable fixed point or a stable limit cycle, depending on the values of the reaction parameters. Wolf & Heinrich then extended the model to n -cells, where the cells are coupled by exchanging molecules with a shared extra-cellular compartment. In [11], the authors show that in a two-cell model, synchronisation with either zero-phase or non-zero phase lag is observed, depending on the reaction parameters chosen. Following the experiments by Richard *et al.*, the authors also mixed two populations of cells which, before mixing, were internally synchronised but out of phase with the other population. Wolf & Heinrich found that, once mixed, the two populations did indeed synchronise, although this took considerably longer to achieve than it did in the experiments. This may suggest that the form of the coupling, or the chemical species chosen to be responsible for the coupling, may not be the correct one. In many models it has been found that the form of the coupling chosen in the model can significantly alter the dynamics observed, as found in [12, 13].

Another system in which synchronisation is often studied involves oscillations in the concentration of adenosine 3', 5'-cyclic monophosphate (*cAMP*) in the amoeba *Dictyostelium discoideum* [2, 14]. These social amoeba feed on bacteria in the forest soil. During the onset of starvation, the cells alter their behaviour to aggregate, and become able to relay *cAMP* signals, in the form of oscillations. In this way, populations of the *Dictyostelium* cells form large aggregates. This process culminates with the formation of a fruiting body which can disperse spores, leading back to the start of the life cycle [2]. One reason why this is much studied is that many of the components involved in the *cAMP* signalling have corresponding components in mammalian pathways, hence the de-

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sire to understand this system in more detail [15].

In this paper we will present an approach to the investigation of synchronisation in models of biochemical processes which is analytic and where the models are stochastic in nature. The vast majority of studies of such systems found in the literature are described by systems of ordinary differential equations (ODEs), which show limit cycle behaviour. However it is now well established that stochastic effects will be significant even in systems containing a large number of molecules [5, 16, 17]. Stochastic oscillations will not only occur at a single frequency, as for limit cycles, and this will have consequences when investigating synchronisation of these oscillations. What studies there have been of synchronisation of stochastic oscillations have been purely numerical [18], and have not considered the possibility of phase lags between oscillations in different cells. However, the key aspects of synchronisation in stochastic biochemical models become particularly clear when an systematic analytical treatment is given.

The outline of the paper is as follows. In Section II we will introduce the formalism we shall be using and give a simple example to illustrate its use. A model of *cAMP* oscillations in *Dictyostelium discoideum* will be analysed in Section III, to show the application of our methods to a more realistic, multi-cell model. In particular, we show under which conditions a phase lag between oscillations in neighbouring cells is introduced. We end with a summary and suggestions for further work.

II. METHODOLOGY

In this section we will describe the formulation of the process in terms of a stochastic model, and then go on to describe the methods we use to see if synchronisation occurs. Fortunately, the quantities we need to calculate to investigate the question of synchronisation are the same as those required to study stochastic oscillations, and so in many cases much of the calculation will already have been carried out.

A. Formalism

The reaction system is described by a chemical master equation, which is written in terms of the integer populations of the chemical species present in the model, which we write as $\mathbf{n} = (n_1, n_2, \dots, n_K)$, if there are K species present in the model. This describes the time evolution of the probability for the system to be in state \mathbf{n} at time t , which is written as $P_{\mathbf{n}}(t)$. Transitions from one state to another are caused by the chemical reactions. We write $T(\mathbf{n}'|\mathbf{n})$ as the transition rate from state \mathbf{n} to state $\mathbf{n}' = \mathbf{n} - \boldsymbol{\nu}$, where $\boldsymbol{\nu}_{\mu} = (\nu_{1\mu}, \dots, \nu_{L\mu})$ is the stoichiometric vector corresponding to reaction μ . The

master equation then has the general form [19, 20]

$$\frac{dP_{\mathbf{n}}(t)}{dt} = \sum_{\mu} T_{\mu}(\mathbf{n}|\mathbf{n} - \boldsymbol{\nu}_{\mu})P_{\mathbf{n}-\boldsymbol{\nu}_{\mu}}(t) - T_{\mu}(\mathbf{n} + \boldsymbol{\nu}_{\mu}|\mathbf{n})P_{\mathbf{n}}(t) \quad (1)$$

To make progress analytically, an approximation scheme must be employed. The crudest scheme is to ignore fluctuations completely, and simply write down an equation for the average

$$\langle n_i(t) \rangle \equiv \sum_{\mathbf{n}} n_i P_{\mathbf{n}}(t), \quad i = 1, \dots, K. \quad (2)$$

In the limit where both the number of molecules in the system and its volume, V , becomes large, we describe the system in terms of the concentrations of the chemical species, $x_i = \lim_{V \rightarrow \infty} \langle n_i \rangle / V$. From Eqs. (1) and (2) one finds the macroscopic, deterministic, equation for $x_i(t)$:

$$\frac{dx_i}{dt} = A_i(\mathbf{x}), \quad A_i(\mathbf{x}) \equiv \sum_{\mu} \nu_{i\mu} f_{\mu}(\mathbf{x}), \quad i = 1, \dots, K, \quad (3)$$

where $f_{\mu}(\mathbf{x})$ is defined by [20]

$$f_{\mu}(\mathbf{x}) = \lim_{V \rightarrow \infty} T_{\mu}(\langle \mathbf{n} \rangle + \boldsymbol{\nu}_{\mu} | \langle \mathbf{n} \rangle) / V. \quad (4)$$

It is straightforward to calculate the function $A_i(\mathbf{x})$ from the chemical reactions and the reaction rates, and so we can determine whether at large times the system tends to a fixed point, a limit cycle, or some other, more complex, type of behaviour.

However, as mentioned already, oscillations seen in biochemical reactions may be stochastic in nature, and so not be described by the deterministic equation (3). To study these we cannot completely ignore the dynamics of the fluctuations contained in Eq. (1). The simplest way of doing this is to simply include stochastic effects (or ‘noise’) as linear deviations from the deterministic result. This is the linear noise approximation (LNA) and is especially simple to implement if the system has reached a stationary state which corresponds to a fixed point of the deterministic equation (3). The LNA then consists of working with the variables $x_i(t)$, writing them as $x_i = x_i^* + V^{-1/2}\xi_i$. The LNA assumes that V is large, so we keep only linear terms in ξ_i when they are substituted into the master equation (1). Here the asterisk signifies a fixed point of Eq. (3) and the $V^{-1/2}$ reflects the Gaussian nature of the approximation [19]. The equation satisfied by the linearised fluctuations ξ is found to be (for details, we refer the reader to the literature [5, 16, 20])

$$\frac{d\xi_i}{dt} = \sum_{j=1}^K M_{ij}\xi_j + \eta_i(t), \quad i = 1, \dots, K, \quad (5)$$

where $\eta_i(t)$ is a Gaussian noise term with zero mean and with correlator $\langle \eta_i(t)\eta_j(t') \rangle = B_{ij}\delta(t - t')$. The matrix M is the Jacobian at the fixed point and the matrix B

can be calculated in the same way that the function A was:

$$\begin{aligned} M_{ij} &= \sum_{\mu} \nu_{i\mu} \left. \frac{\partial f_{\mu}}{\partial x_j} \right|_{\mathbf{x}=\mathbf{x}^*}, \\ B_{ij} &= \sum_{\mu} \nu_{i\mu} \nu_{j\mu} f_{\mu}(\mathbf{x}^*), \quad i, j = 1, \dots, K. \end{aligned} \quad (6)$$

Since Eq. (5) describes fluctuations about the stationary state and is linear, we can analyse the equation using Fourier transforms. Denoting the temporal Fourier transform of $\xi_i(t)$ as $\tilde{\xi}_i(\omega)$, we may write Eq. (5) as $\tilde{\xi}_i(\omega) = \sum_j \Phi_{ij}^{-1}(\omega) \tilde{\eta}_j(\omega)$ where $\Phi_{ij} \equiv -M_{ij} - i\omega \delta_{ij}$. This then allows us to calculate the power spectral density matrix (PSDM)

$$P_{ij}(\omega) \equiv \langle \tilde{\xi}_i(\omega) \tilde{\xi}_j^*(\omega) \rangle = \sum_{l=1}^K \sum_{m=1}^K \Phi_{il}^{-1}(\omega) B_{lm} (\Phi^{\dagger})_{mj}^{-1}(\omega). \quad (7)$$

In earlier work on analysing the nature of the stochastic oscillations about a fixed point, only the diagonal entries of this matrix (the ‘power spectra’ P_{ii}) were of interest [5, 21]. Here we shall also be interested in the off-diagonal entries, but as mentioned in the Introduction, if Eq. (7) has been calculated so as to obtain the power spectrum, the off-diagonal elements can be immediately read-off.

An application quite similar to the one we are discussing here is that described by Rozhnova *et. al.* who used the off-diagonal elements to show how stochastic oscillations can become synchronised [22]. They constructed an epidemiological model of a network of cities, where a disease can be transported between cities due to movement of infected commuters. We will use a similar approach but instead of cities and people, we will work with cells and molecules. Unlike the diagonal elements — the power spectra — the off-diagonal elements of the PSDM will in general be complex. Often, these elements are normalised, by using the relevant power spectra. This quantity is then known as the complex coherence function (CCF) [22–24],

$$C_{ij}(\omega) = \frac{P_{ij}(\omega)}{\sqrt{P_{ii}(\omega)P_{jj}(\omega)}}. \quad (8)$$

As this is a complex function, it can be expressed in terms of a magnitude and a phase,

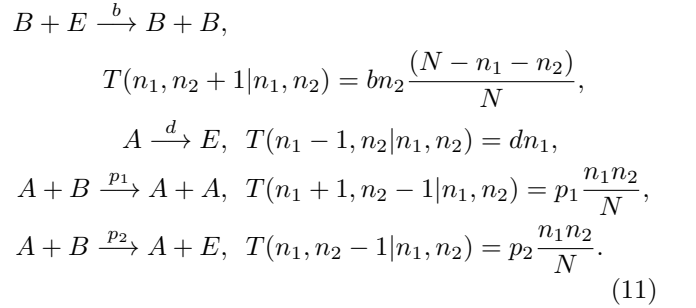
$$|C_{ij}(\omega)| = \frac{|P_{ij}(\omega)|}{\sqrt{P_{ii}(\omega)P_{jj}(\omega)}}, \quad (9)$$

$$\phi_{ij}(\omega) = \arctan\left[\frac{\text{Im}(C_{ij}(\omega))}{\text{Re}(C_{ij}(\omega))}\right] = \arctan\left[\frac{\text{Im}(P_{ij}(\omega))}{\text{Re}(P_{ij}(\omega))}\right]. \quad (10)$$

The magnitude of the CCF tells us the similarity between two signals, as a function of ω . The phase of the CCF tells us the phase lag between two signals as a function of the frequency ω [24]. For example, if the two signals are in phase the CCF will be a real function. To clarify the use of these concepts we apply them to a simple example.

B. Simple example

The biochemical reaction scheme we will use to illustrate the methodology is a toy system, but many more complex reaction schemes contain molecular species which behave in this way. The system consists of two species A and B , which may both lose molecules through diffusion out of the cell and also gain them through diffusion into the cell. Species A requires molecules of species B to sustain its numbers, and they become depleted when too many B molecules have been used up. This is essentially a predator-prey dynamics, and we will use this language to discuss it, since it makes the results more intuitive. The sustained stochastic oscillations have been previously investigated using the formalism described in Section II A [21]. The actions of these species is described by the following interactions and their corresponding transition rates:



The variable E is introduced to give the system a maximum carrying capacity, which is the ‘size of the system’ N . That is, the total number of individuals at any time is fixed, $n_1 + n_2 + n_E = N$, where n_1, n_2 and n_E are, respectively, the numbers of A, B and E molecules in the cell. Because of this we will use N as the large parameter employed in the LNA in this example. We have also only allowed for species A to diffuse out of the cell, and for species B to diffuse in (subject to existing concentration). The relation $n_E = N - n_1 - n_2$ has been used to simplify the transition rates above.

Applying the methodology described in Section II A, the deterministic equations (3) are

$$\begin{aligned} \frac{dx_1}{dt} &= p_1 x_1 x_2 - dx_1, \\ \frac{dx_2}{dt} &= bx_2(1 - x_1 - x_2) - (p_1 + p_2)x_1 x_2. \end{aligned} \quad (12)$$

To go beyond this, we use the LNA which is fully characterised by the two matrices M and B , which can be calculated from Eq. (6), and which are given explicitly in [21].

The macroscopic equations have a single nontrivial stable fixed point (or ‘steady state’), and for the reaction parameters chosen here the eigenvalues of the Jacobian, evaluated at the fixed point, have an imaginary component and sustained stochastic oscillations can be observed

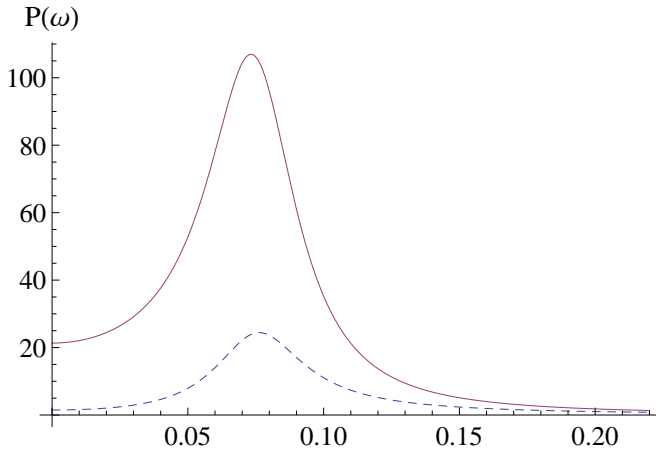


FIG. 1. Power spectra of the oscillations observed in the two-species example calculated using the LNA. The red (solid) curve is for the prey and the blue (dashed) curve for the predator. The parameter values used are: $b = 0.1$, $d = 0.1$, $p_1 = 0.25$, $p_2 = 0.05$, and $N = 3200$.

around the fixed point [21]. This is illustrated in Figure 1 which shows the power spectra of the oscillations, calculated using Eq. (7) for a particular choice of the reaction parameters. The large peak shows the existence of amplified stochastic oscillations for a reasonably narrow range of values about a characteristic frequency [21].

We use the complex coherence function (CCF), introduced in the previous section, to study the relation between these two oscillators. In general, the CCF will be a complex-valued function. Figure 2 shows the magnitude of the CCF, showing a strong shared signal in the two oscillators. The analytical result obtained from Eq. (9), is compared with results from numerical simulations, obtained using the Gillespie algorithm [25]. The phase lag, defined in Eq. (10), is found to have the simple form

$$\phi(\omega) = \tan^{-1} \left[\frac{\alpha\omega}{\beta + \gamma\omega^2} \right], \quad (13)$$

for this two species model. Here α, β and γ are calculable combinations of the parameters b, d, p_1 and p_2 defining the system. The phase lag is shown in Figure 3 along with simulation results. Figure 1 indicates that the oscillations are most significant over the frequency range $0.05 \leq \omega \leq 0.11$. The phase tends to π as $\omega \rightarrow 0$ and $\omega \rightarrow \infty$. However within the range $0.05 \leq \omega \leq 0.10$ the phase lag remains fairly constant, at around 2 radians.

We end this section by remarking that we do not use the word synchronisation to describe the phenomena observed in this section. We will only use this term for the interaction between self-sustained oscillators. We do not have two self-sustained oscillators here, as neither species would continue to oscillate if the other were removed or held fixed. In the next section, we will look at a more realistic, multi-cellular model, where self-sustained oscillators in neighbouring cells become synchronised due to

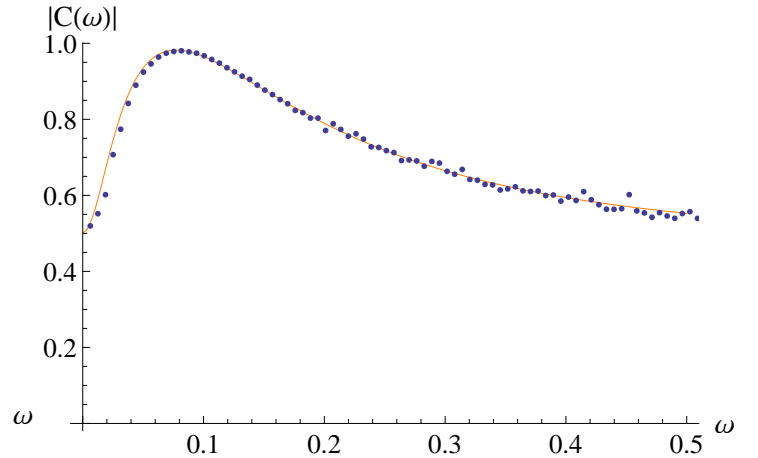


FIG. 2. Magnitude of the CCF $C_{12}(\omega)$ in the predator prey model. The orange curve is the theoretical prediction and the blue dots are the results obtained from numerical simulation.

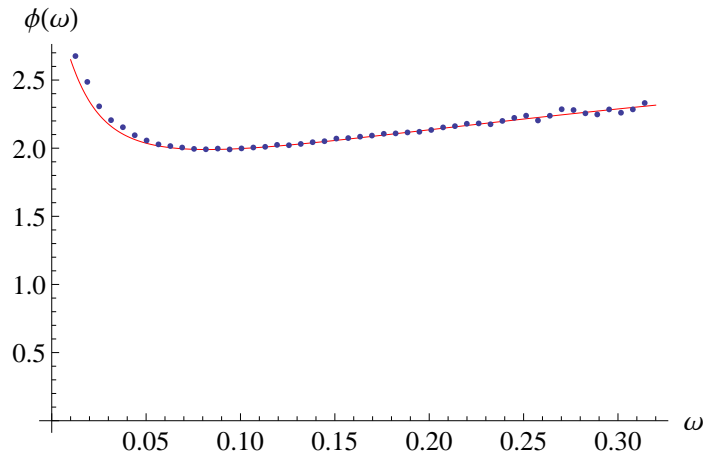


FIG. 3. The phase spectra in the predator prey model, showing the phase lag (in radians) between the oscillators as a function of ω . The red line is the theoretical prediction and the blue dots are the results obtained from numerical simulation.

the coupling between the cells. This is what we mean by the ‘weak interaction’ between oscillators.

III. COLLECTIVE BEHAVIOUR IN *DICTYOSTELIUM DISCOIDEUM*

The reaction system we will now discuss is a model of oscillations in the concentration of adenosine 3', 5'-cyclic monophosphate (*cAMP*), which are observed within the amoeba *Dictyostelium discoideum*. The model is due to Kim *et. al.* and in their work was used to study the synchrony of stochastic oscillations in the concentration of *cAMP* across multiple cells *via* numerical simulation.

Previously, the pathway that produces these oscillations had been modelled using a set of non-linear ODEs, which exhibit limit cycle behaviour [2]. Starting from such a model, described in [26], Kim *et. al.* [18] show that considering stochastic oscillations, in addition to those in the limit cycle regime, increases the robustness of the oscillations. That is, oscillations, whether deterministic or stochastic in origin, are observed over a larger volume of parameter space than would be the case if only deterministic oscillations were considered. Kim *et. al.* also demonstrate that, in the deterministic model, the oscillations cease to be observed if the reaction parameter values are slightly altered. The robustness of the oscillations in the model is desirable, as any parameter values present in a mathematical model of a biochemical reaction system will have an associated uncertainty. In addition to this, because the physical system involves an aggregation of cells, conditions (*e.g.* temperature) may vary from cell to cell, so the reaction parameters may not be identical inside each cell. Because of this, Kim *et. al.* made small perturbations to the reaction parameters in different cells to see if oscillations were still observed, and whether they still synchronised.

In this section we will use the theoretical framework, outlined in Section 2, to obtain analytical results for the system in terms of the CCF. This provides information on the strength of coupling between the oscillations in neighbouring cells (through the magnitude of the CCF),

as well as the phase lag between the oscillations, if any. The possibility of a phase lag, introduced due to the heterogeneity of the reaction parameters in different cells was not considered in [18]. These calculations will be performed whilst parameters in the model, such as reaction rates and cell volumes, are varied, to identify the impact of changing these parameters. To study these multi-cell models in a stochastic framework we will use some ideas from previous work, where the LNA was applied to multi-compartment biochemical models [27].

Although we will be interested in a multi-cell model, we will begin with a one-cell model, which consists of a cell and an extra-cellular environment. The *cAMP* within the cell is denoted by *cAMP_i*, the *cAMP* in the extra-cellular environment is denoted by *cAMP_e*. The other components present in the model are adenylyl cyclase (ACA), protein kinase (PKA), mitogen-activated protein kinase (ERK2), the *cAMP* phosphodiesterase (RegA) and the ligand-bound cell receptor (CAR1). The coupling between the two compartments is caused by the external *cAMP* binding to the cell receptor. To label the species we use: (ACA, PKA, ERK2, RegA, *cAMP_i*, *cAMP_e*, CAR1) = ($x_1, x_2, x_3, x_4, x_5, x_6, x_7$). The reaction scheme, together with the reaction kinetics and numerical values of the reaction rates are given in Table 1, which can be used to calculate the matrices *M* and *B* using Eq. (6).

| Reaction Scheme | | | |
|---|---------------------|------------------|---|
| Reaction | $f_\mu(\mathbf{x})$ | $\nu_{i\mu}$ | Rate Constant Value |
| $CAR1 \xrightarrow{k_1} ACA + CAR1$ | $k_1 x_7$ | $\nu_{11} = +1$ | $k_1 = 1.96 \text{min}^{-1}$ |
| $ACA + PKA \xrightarrow{k_2} PKA$ | $k_2 x_1 x_2$ | $\nu_{12} = -1$ | $k_2 = 0.882 \mu\text{M}^{-1} \text{min}^{-1}$ |
| $cAMP_i \xrightarrow{k_3} PKA + cAMP_i$ | $k_3 x_5$ | $\nu_{23} = +1$ | $k_3 = 2.55 \text{min}^{-1}$ |
| $PKA \xrightarrow{k_4} \emptyset$ | $k_4 x_2$ | $\nu_{24} = -1$ | $k_4 = 1.53 \text{min}^{-1}$ |
| $CAR1 \xrightarrow{k_5} ERK2 + CAR1$ | $k_5 x_7$ | $\nu_{35} = +1$ | $k_5 = 0.588 \text{min}^{-1}$ |
| $PKA + ERK2 \xrightarrow{k_6} PKA$ | $k_6 x_2 x_3$ | $\nu_{36} = -1$ | $k_6 = 0.816 \mu\text{M}^{-1} \text{min}^{-1}$ |
| $\emptyset \xrightarrow{k_7} RegA$ | k_7 | $\nu_{47} = +1$ | $k_7 = 1.02 \mu\text{M} \text{min}^{-1}$ |
| $ERK2 + RegA \xrightarrow{k_8} ERK2$ | $k_8 x_3 x_4$ | $\nu_{48} = -1$ | $k_8 = 1.274 \mu\text{M}^{-1} \text{min}^{-1}$ |
| $ACA \xrightarrow{k_9} cAMP_i + ACA$ | $k_9 x_1$ | $\nu_{59} = +1$ | $k_9 = 0.306 \text{min}^{-1}$ |
| $RegA + cAMP_i \xrightarrow{k_{10}} RegA$ | $k_{10} x_4 x_5$ | $\nu_{510} = -1$ | $k_{10} = 0.816 \mu\text{M}^{-1} \text{min}^{-1}$ |
| $ACA \xrightarrow{k_{11}} cAMP_e + ACA$ | $k_{11} x_1$ | $\nu_{611} = +1$ | $k_{11} = 0.686 \text{min}^{-1}$ |
| $cAMP_e \xrightarrow{k_{12}} \emptyset$ | $k_{12} x_6$ | $\nu_{612} = -1$ | $k_{12} = 4.998 \text{min}^{-1}$ |
| $cAMP_e \xrightarrow{k_{13}} CAR1 + cAMP_e$ | $k_{13} x_6$ | $\nu_{713} = +1$ | $k_{13} = 22.54 \text{min}^{-1}$ |
| $CAR1 \xrightarrow{k_{14}} \emptyset$ | $k_{14} x_7$ | $\nu_{714} = -1$ | $k_{14} = 4.59 \text{min}^{-1}$ |

TABLE I. The reactions and their associated rate constants, given in micromoles and minutes. All the reactions have mass-action kinetics. Only non-zero entries of the stoichiometry matrix, $\nu_{i\mu}$, are shown.

Some results from the one-cell model will be shown,

before looking at the two cell model. The reaction pa-

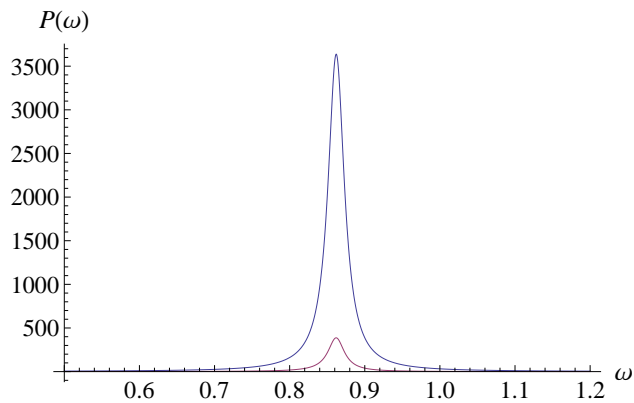


FIG. 4. Theoretical power spectra for the internal (larger peak) and external *cAMP* in the one-cell model. The peak is around $\omega = 0.83$. The reaction rates are given in Table 1. In [18] the cell volume was chosen to be 3.672×10^{-14} l.

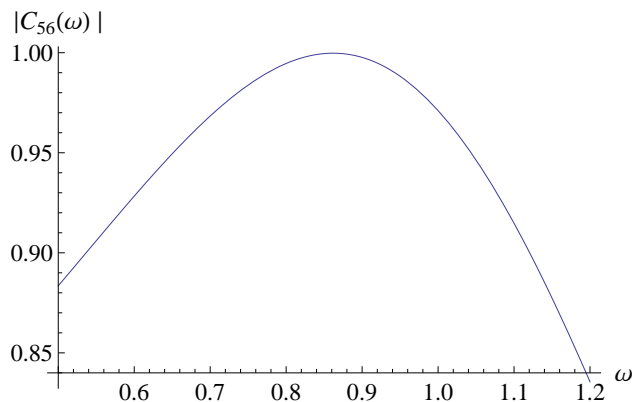


FIG. 5. Magnitude of the (theoretical) CCF for the internal and external *cAMP* fluctuations in the one-cell model. The reaction rates are given in Table 1.

parameters are chosen to be those which yielded the steady state in Figure 1B in [18]. As in Section 2, we use the linear noise approximation to quantify the fluctuations around the steady state. We can then use Eq. (7) to calculate the PSDM and Eq. (8) to calculate the CCF. The specific form of the analytic results are not very illuminating and we content ourselves with showing the results graphically. Figure 4 shows the power spectra for the internal and external *cAMP*. The values of the reaction parameters are given in the caption. Figure 5 shows the magnitude of the CCF for these two species, showing a very strong correlation, especially in the frequency range within which the oscillations are significant. The CCF is displayed parametrically in Figure 6. It has both real and imaginary components, which indicates a phase lag.

In [18], the authors construct models of many cells, which are all coupled *via* the external *cAMP*. A diagram of such a model, with three cells, is shown in Figure 5A

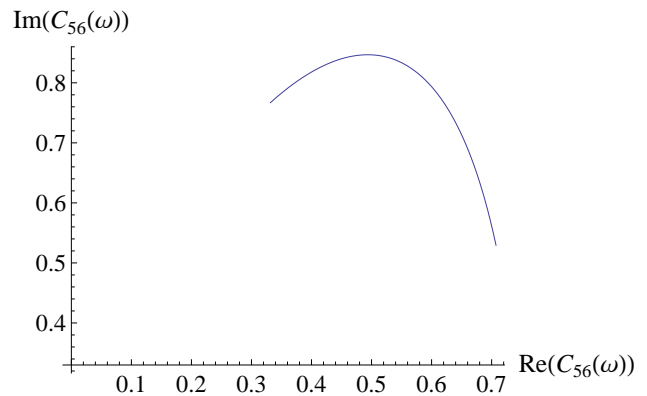


FIG. 6. Parametric plot of the (theoretical) CCF for the internal and external *cAMP* fluctuations in the one-cell model. Only results for $0.5 \leq \omega \leq 1.2$ are shown. The reaction rates are given in Table 1.

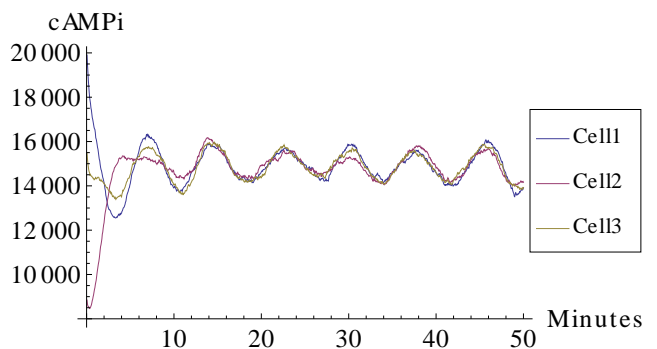


FIG. 7. Time series for *cAMPi* (in particle numbers) in different cells for a three-cell model, generated using the Gillespie algorithm. The three species are given different initial conditions, but within a short amount of time they oscillate in phase with each other around their common fixed point.

of [18]. If the reaction parameters within each cell are identical, the oscillations in the three cells become synchronised with zero-phase lag. This effect can be easily seen by looking at the time series. This is shown for a three-cell model in Figure 7. To apply our analysis to a multi-cell model we proceed as we did for the one cell model: for an n cell model there will be $6n + 1$ variables.

Synchronisation with a non-zero phase lag can be found when the reaction parameters differ from cell to cell. We illustrate this with a two cell model, where the reaction parameters in one cell are those used for the single cell model, and the reaction parameters in the second cell are obtained by making random perturbations to the original parameters. Below the relation between the internal *cAMP* oscillations in each cell is examined, for one such parameter choice. Figure 8 shows the power spectra for these two species. Figures 9 & 10 show the

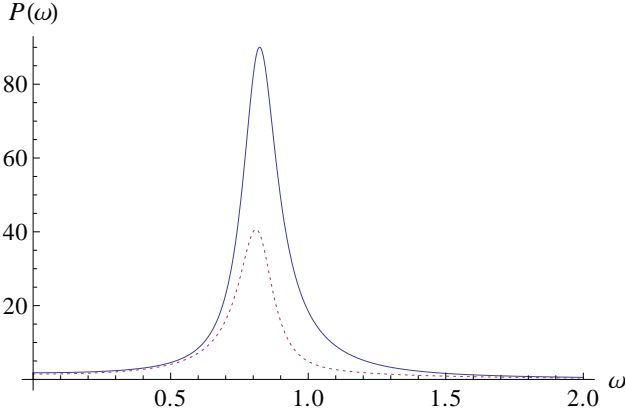


FIG. 8. Theoretical power spectra for *cAMPi* in each cell of the two-cell model. The dashed line corresponds to the oscillations in the second cell, for which the reaction parameters are: $k_1 = 2.3\text{min}^{-1}$, $k_2 = 0.96\mu\text{M}^{-1}\text{min}^{-1}$, $k_3 = 2.1\text{min}^{-1}$, $k_4 = 1.9\text{min}^{-1}$, $k_5 = 0.4\text{min}^{-1}$, $k_6 = 0.72\mu\text{M}^{-1}\text{min}^{-1}$, $k_7 = 0.7\mu\text{Mmin}^{-1}$, $k_8 = 1.05\mu\text{M}^{-1}\text{min}^{-1}$, $k_9 = 0.26\text{min}^{-1}$, $k_{10} = 0.89\mu\text{M}^{-1}\text{min}^{-1}$, $k_{11} = 0.46\text{min}^{-1}$, $k_{13} = 15\text{min}^{-1}$, $k_{14} = 5.8\text{min}^{-1}$. The parameter k_{12} was set to 9min^{-1} .

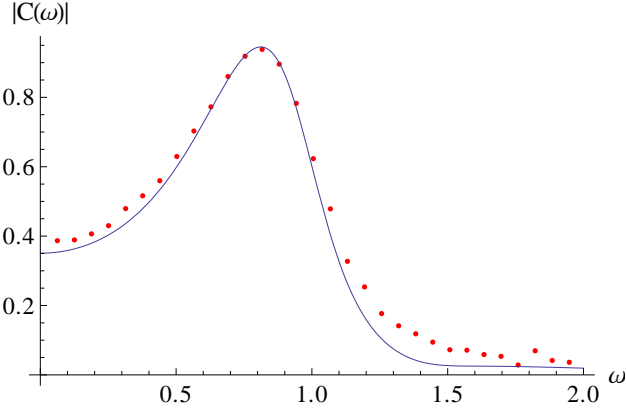


FIG. 9. The magnitude of the CCF for the *cAMPi* in each cell of the two-cell model. The solid line is the theoretical result, the dots are from 6000 numerical simulations

relevant CCF for these two species, whilst Figure 11 displays the phase lag present, calculated using Eq. (10). The theoretical results show good agreement with those from numerical simulation. Although the reaction parameters are different in each cell, there remains a strong shared signal in the oscillations.

Throughout this section so far, we have assumed that all of the cells have the same volume. We will now look at the consequences of relaxing this restriction, which could be due to natural variation in the cell size. We find that a phase lag is introduced when the cells are of different sizes. Given that, for this particular system, it is desirable to have in phase synchronisation, it is important to see how large these phase lags are for various differ-

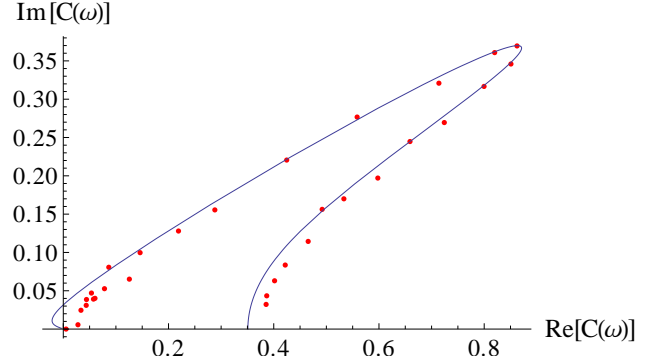


FIG. 10. Parametric plot of the CCF for the *cAMPi* in each cell of the two-cell model. The solid line is the theoretical result, the dots are from 6000 numerical simulations. Results are shown for $0 \leq \omega \leq 3$.

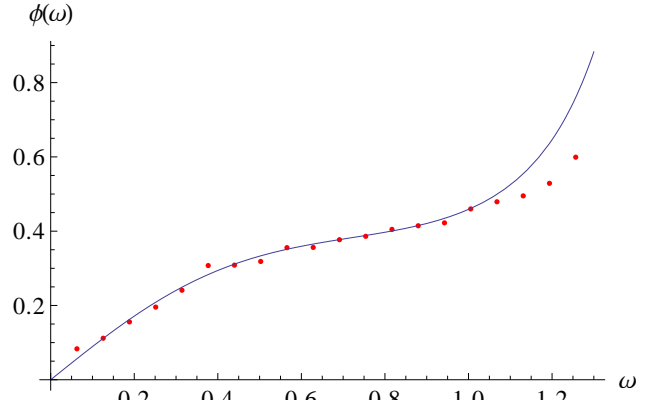


FIG. 11. Phase lag for oscillations of *cAMPi* in each cell for the two-cell model. The solid line is the theoretical result, the dots are from 6000 numerical simulations. Over the frequency range where oscillations are significant, the phase lag remains fairly constant.

ences in cell size. To do this, we again studied a two-cell model, in which we fixed the volume of one cell, at the volume used previously which we will call V_I , and the extracellular region (fixed at $5V_I$), and varied the volume of the other cell. The reaction parameters in each cell were identical and were chosen to be those used for the one cell model. The parameter governing the rate of degradation of *cAMPe*, k_{12} , was set to 12min^{-1} . Figure 12 shows details of the CCF for the case where the second cell has volume $1.4V_I$. The parametric plot shows that the imaginary component of the CCF is much smaller than the real component: this means that the phase lag is very small. We then increased the volume of the second cell to $2V_I$. Figure 13 shows that the imaginary part of the CCF is larger than before, indicating a more significant phase lag. The phase spectrum for this system is shown in Figure 14. At the frequency for which the oscillations are most significant (the frequency for which

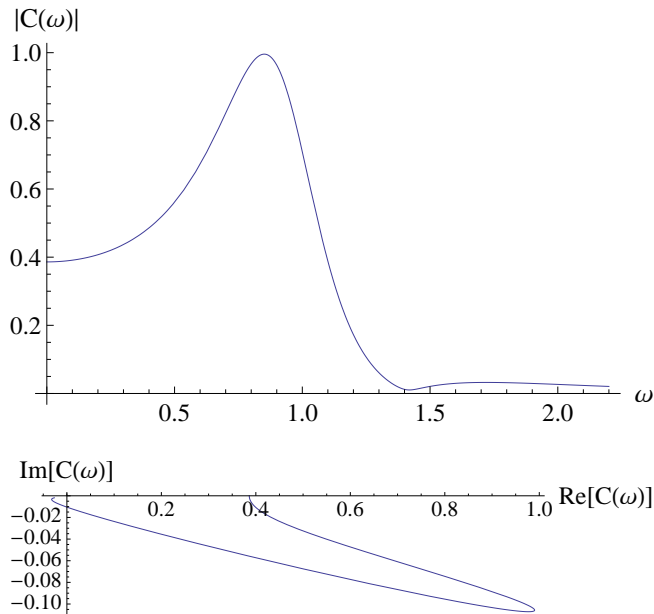


FIG. 12. Results for a two-cell model, where the cell volumes are not identical. Here, the cell volumes were chosen to be V_I and $1.4V_I$, where V_I is the cell volume chosen by Kim. *et. al.*. The reaction parameters were the same in each cell, and were those used for the one-cell model. Top: The magnitude of the CCF for the *cAMP* fluctuations in each cell. Bottom: A parametric plot, showing the real and complex parts of the same CCF for the frequency range $0 \leq \omega \leq 2$. A small phase lag has appeared, due to the different cell volumes.

the power is greatest), the phase lag is about 0.2 radians, roughly 11° . This shows that quite large differences in volume are required to introduce measurable phase lags: varying the cell size by a few percent does not have a significant effect. This is positive from the point of view of in-phase synchronisation being robust in this system. We also repeated this analysis whilst varying the volume of the extracellular region. However, this did not produce significantly different results from those presented here.

In this section we have studied a model of *cAMP* oscillations in *Dictyostelium*, due to Kim *et. al.* Using the theoretical framework of the LNA, we have shown how analytical results can complement the numerical results which have already been obtained from this model [18]. The analysis performed in this section extends naturally to models containing many cells. However, in reality the *Dictyostelium* cells can form aggregates containing thousands of cells. In this case, the distances between cells will be much larger, and the diffusion speed of the molecules would need to be taken into consideration. A spatial model would be required to capture this effect, although the mechanism by which the cells synchronise would be the same as the one outlined here.

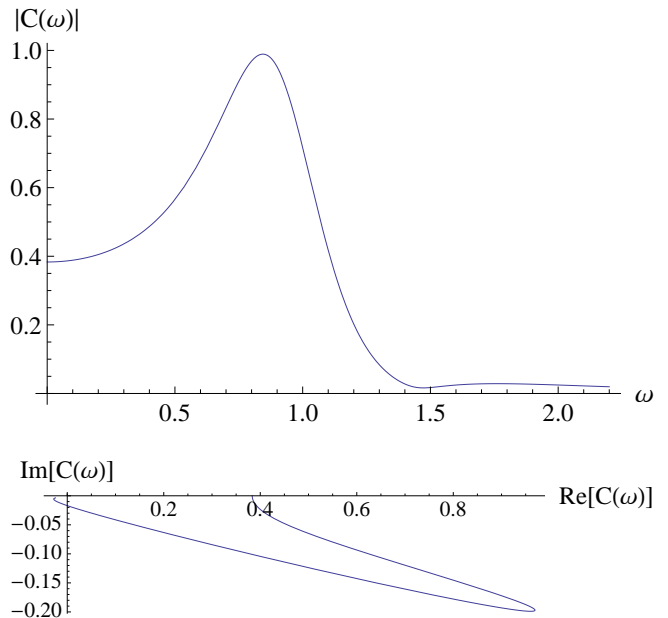


FIG. 13. Results for a two-cell model, where the cell volumes are not identical. Here, the cell volumes were chosen to be V_I and $2V_I$, where V_I is the cell volume chosen by Kim. *et. al.*. The reaction parameters were the same in each cell, and were those used for the one-cell model. Top: The magnitude of the CCF for the *cAMP* fluctuations in each cell. Bottom: A parametric plot, showing the real and complex parts of the same CCF for the frequency range $0 \leq \omega \leq 2$. A phase lag has appeared, due to the different cell volumes.

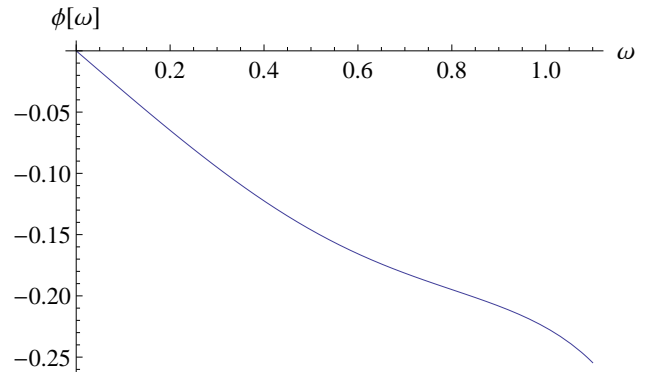


FIG. 14. The phase spectrum for the *cAMP* fluctuations in each cell in a two-cell model for the case when the cell volumes are not identical. The cell volumes were V_I and $2V_I$. The power spectra for these oscillations peak at $\omega = 0.9$. The phase lag for oscillations of this frequency is 0.2 radians.

IV. DISCUSSION

In this work we have shown how stochastic oscillations in biochemical models can synchronise, and how analytical expressions for this effect may be found. Comparisons between numerical results and theoretical predictions for the CCF (and related quantities) were found to

be good, especially in frequency ranges where the oscillations were significant. There have been very few models devised which study the synchronisation of stochastic oscillations across multiple cells, and those which have been constructed, such as [18], are examined using numerical simulation. We hope that the analytical work described here can complement the numerical approach. In our study of the model of *cAMP* signalling, we found that the stochastic oscillations across cells strongly influenced each other, and oscillations of species in different cells synchronised rapidly, as illustrated in Figure 7. In the case where the cells were of equal volume, and the reaction parameters across cells were the same, the oscillations synchronised in phase. Introducing changes to the reaction parameters in different cells, or making the cell volumes different to each other introduced a phase lag. These findings are similar to those reported in [22], in the context of epidemiological modelling. However, quite large changes had to be made before the lag was found to be significant. These results are positive for in-phase synchronisation being robust in this system. One slightly surprising result we found was that the size of the phase-lag was unaffected by varying the volume of the extracellular compartment. This is probably not realistic, and could be due to the assumption that all compartments are well mixed. For a larger extracellular compartment, communication between cells would be expected to take longer, which could affect the phase lag.

The analysis used to study the model of *cAMP* oscillations was also applied to a model of oscillations in

yeast glycolysis, due to Wolf & Heinrich [11]. Although not presented here, very different results were obtained for this model. The magnitude of the CCF for oscillators in different cells was extremely small. Using the reaction parameters chosen in the original article, we obtained CCFs with a magnitude of ≤ 0.01 over the relevant frequency range. This indicates that the shared signal in the oscillators is extremely weak. The magnitude of the CCF remained small throughout an extensive parameter search of the model. This weak coupling between the cells could explain why, in the deterministic framework, the oscillators take a longer-than-expected time to synchronise. The relation between the time to synchronise and the magnitude of the CCF could be an interesting avenue for further work. The magnitude of the fluctuations, here governed by the system-size, could also influence whether synchronisation is observed *i.e.* if the system-size is small (and therefore the fluctuations are relatively large), is the synchronisation of two oscillators observed if the magnitude of the corresponding CCF has low or intermediate value? Such questions we leave for the future. Our aim here has been to formulate the analytic study of synchronisation of stochastic oscillators in a biochemical context, and to illustrate its use in a specific model. We hope that this will encourage others use these techniques to investigate other biochemical systems.

ACKNOWLEDGEMENTS

JDC acknowledges support from the EPSRC and Program Prin2009 financed by the Italian MIUR.

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